Effects of Watercress Consumption on Metabolism of a Tobacco-specific Lung Carcinogen in Smokers[1]

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Abstract

Epidemiological studies indicate that vegetable consumption protects against lung cancer in humans, but the protective constituents have not been identified. Phenethyl isothiocyanate (PEITC), which is released upon chewing of watercress (*nasturtium officinale*), is a chemopreventive agent against lung cancer induced by the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rats and mice. PEITC inhibits the carcinogenicity of NNK by inhibiting its metabolic activation and thereby increasing the levels of detoxified metabolites excreted in urine. In this study, our goal was to determine whether watercress consumption would modify NNK metabolism in smokers. Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables and other sources of isothiocyanates throughout the study. They donated 24-h urine samples on 3 consecutive days (baseline period). One to 3 days later, they consumed 2 ounces (56.8 g) of watercress at each meal for 3 days and donated 24-h urine samples on each of these days (watercress consumption period). One and 2 weeks later, they again donated 24-h urine samples on 2–3 consecutive days (follow-up periods). The samples were analyzed for two metabolites of NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-Ω-D-glucosiduronic acid (NNAL-Gluc). NNAL-Gluc is believed to be a detoxification product of NNK. The urine samples were also analyzed for PEITC-NAC, a metabolite of PEITC. Minimum exposure to PEITC during the watercress consumption period averaged 19–38 mg/day. Seven of the 11 subjects had increased levels of urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period, compared to the baseline period. Overall, the increase in urinary NNAL plus NNAL-Gluc in this period was significant [mean ± SD 0.924 ± 1.12 nmol/24 h (33.5%); $P < 0.01$]. Urinary levels of NNAL plus NNAL-Gluc returned to near baseline levels in the follow-up periods. The percentage of increase in urinary NNAL plus NNAL-Gluc during days 2 and 3 of the watercress consumption period correlated with intake of PEITC during this period, as measured by total urinary PEITC-NAC ($r = 0.62$; $P = 0.04$). The results of this study support our hypothesis that PEITC inhibits the oxidative metabolism of NNK in humans, as seen in rodents, and support further development of PEITC as a chemopreventive agent against lung cancer. This is the first study to report an effect of vegetable consumption on metabolism of a lung carcinogen in humans.

Introduction

NNK,[3] a potent pulmonary carcinogen in rodents, is believed to be one of the causes of lung cancer in smokers (1, 2). The total dose of NNK required to produce lung tumors in rats is similar to its total uptake during a lifetime of smoking (3, 4). The structure of NNK is illustrated in Fig. 1. NNK requires metabolic activation by α-hydroxylation to express its carcinogenic activity (1, 2, 5–7). The metabolic activation of NNK is catalyzed by cytochrome P-450 enzymes in rodents and humans (8–12). Other enzymes may also be involved. The metabolic activation of NNK generates reactive intermediates that form a variety of DNA adducts that are involved in carcinogenesis (1, 2, 5–7). In rodents and humans, NNK is also extensively converted to its carbonyl reduction product (NNAL), which, like NNK, is a strong pulmonary carcinogen (4, 12–15). NNAL undergoes metabolic activation by α-hydroxylation, producing the same DNA adducts as does NNK (16). NNAL also can be conjugated to NNAL-Gluc, which is believed to be a detoxified metabolite of NNAL and NNK. NNAL and NNAL-Gluc are excreted in the urine of rodents, primates, and humans exposed to NNK (4, 17, 18). If the α-hydroxylation pathways and other oxidative metabolic pathways of NNK and NNAL are inhibited, the result will be increased excretion of NNAL and NNAL-Gluc in urine.

A number of epidemiological studies have shown that vegetable consumption protects against lung cancer in smokers (19). Experimental studies have demonstrated that compounds found in vegetables can prevent tumor induction (20, 21). One such chemopreventive agent is PEITC, which inhibits NNK pulmonary carcinogenesis in rats and mice (22–25). The mechanism of inhibition has been studied in detail. The results of these investigations have clearly shown that PEITC decreases the metabolic activation of NNK by inhibiting cytochrome P-450 enzymes involved in its α-hydroxylation (11, 23,

Received 6/28/95; revised 9/7/95; accepted 9/9/95.

[1] This study was supported by National Cancer Institute Grant CA-46535. Cotinine and creatinine analyses were carried out in the American Health Foundation Clinical Biochemistry Facility, supported in part by National Cancer Institute Cancer Center Support Grant CA-17613.

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[3] The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-O-D-glucosiduronic acid; PEITC, phenethyl isothiocyanate; PEITC-NAC, *N*-acetyl-*S*-(*N*-phenethylthiocarbamoyl)-L-cysteine.

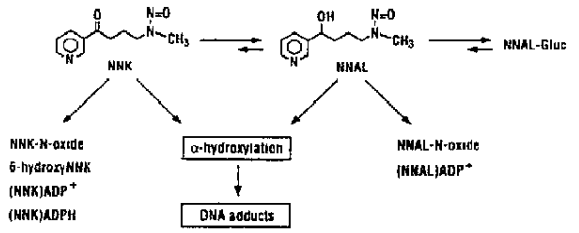

Fig. 1. Overview of NNK metabolism. For details, see Ref. 2, 3, and 5–7.

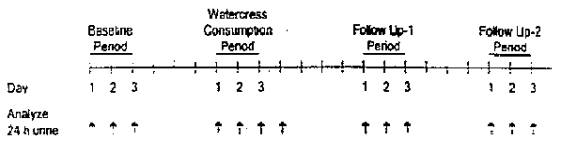

Fig. 2. Protocol for examining the effects of watercress consumption on NNK metabolism in smokers. Number of days between periods varied. See "Materials and Methods" for details.

26–27). Decreases in NNK metabolic activation and lung tumor induction have been observed at nontoxic doses of PEITC administered in the diet (23). In ongoing studies, we have demonstrated that chronic dietary administration of PEITC to rats inhibits the metabolic activation of NNK, with a concomitant increase in urinary excretion of NNAL plus NNAL-Gluc.[4] On the basis of currently available data in laboratory animals, PEITC appears to be a highly promising compound for chemoprevention of lung cancer in addicted smokers.

PEITC occurs naturally as its thioglucoside conjugate gluconasturtiin in a number of cruciferous vegetables (28). Watercress (*nasturtium officinale*) is one common vegetable that is rich in gluconasturtiin (29). Watercress and other cruciferous vegetables contain the enzyme myrosinase, which is separated cellularly from the thioglucoside conjugate. When the vegetable is chewed or otherwise macerated, cellular structure is disrupted, and myrosinase comes into contact with the thioglucoside conjugate and catalyzes its hydrolysis. Thus, upon chewing of watercress, gluconasturtiin is hydrolyzed to PEITC, an isothiocyanate responsible for the sharp taste of this vegetable. Chung *et al.* (30) demonstrated that a minimum of 2–5 mg of PEITC was released in subjects who ate 30 g of watercress. Because PEITC has been shown to inhibit the metabolic activation of NNK in rodents, and substantial amounts of PEITC are released upon consumption of watercress in humans, we hypothesized that NNK metabolism would be modified when smokers ate watercress. We tested this hypothesis in the present study by determining the effects of watercress consumption on two urinary metabolites of NNK: NNAL and NNAL-Gluc. On the basis of studies in rodents and primates, we expected urinary levels of NNAL plus NNAL-Gluc to increase upon watercress consumption.

Materials and Methods

Subjects. Protocols were reviewed and approved by the American Health Foundation Institutional Review Board for protection of human subjects. All subjects signed a consent form before participation. All subjects were healthy smokers.

Protocol. The critical parameter in this study was total NNAL plus NNAL-Gluc in urine. This varies among individuals depending on the number of cigarettes smoked and on metabolic differences (31). Therefore, each subject served as his/her own control during the study.

The protocol is summarized in Fig. 2. In the "baseline period," 24-h urine samples were collected beginning with the first morning void on 3 consecutive days. The "watercress consumption period" commenced 1–4 days later. The watercress was purchased at a local supermarket on the day before the watercress consumption period, and each 2-ounce (56.8-g) portion was provided to the subjects in plastic ZIP-lock bags. Subjects consumed 2 ounces of watercress at each meal for 3 days, beginning with breakfast on the first day. Twenty-four-h urine samples were collected beginning with the first morning void on each of the 3 watercress consumption days and on the subsequent day. Eight of the 11 subjects also collected 24-h urine samples in "follow-up period 1," which was 3 consecutive days, 1 week after the watercress consumption period. Five of the subjects collected 24-h urine samples in "follow-up period 2," which was 3 consecutive days, 2 weeks after the watercress consumption period.

The protocol was carried out in 3 segments: March 1994 for subjects 5 and 6; August 1994 for subjects 1–4; and February to March 1995 for subjects 7–11. Subjects 5 and 6 collected urine only during the baseline and watercress consumption periods because this was an initial test to determine whether any effect of watercress would be seen. For subjects 1–4, follow-up period 1 period was introduced to determine whether the effect was transitory. Subject 1 was unable to complete follow-up period 1 due to a death in the family. For subjects 7–11, follow-up period 2 was introduced to determine whether watercress consumption might have long-lasting effects, as suggested by the data for subjects 2 and 3 in follow-up period 1.

Each subject was asked to smoke a constant number of cigarettes per day ± 2 throughout the entire study. They recorded the number of cigarettes smoked on each day of the study. Subjects were asked not to eat cruciferous vegetables during the entire study, with the exception of watercress on the prescribed days. Cruciferous vegetables excluded were broccoli, cabbage (including cole slaw and sauerkraut), cauliflower, brussels sprouts, radishes, turnips, arugula, mustard greens, and collard greens. Also excluded were other potential sources of isothiocyanates including mustard, salad dressing (except oil and vinegar), and mayonnaise. Permissible vegetables included potatoes, corn, peas, lettuce, cucumbers, carrots, peppers, beans, asparagus, tomatoes, onions, beets, celery, squash, and spinach. All subjects kept a record of everything eaten during the study.

Collection and Analysis of Urine. Twenty-four-h urine samples were collected in amber bottles containing ammonium sulfamate (4). NNAL and NNAL-Gluc were determined by gas chromatography with detection by a Thermal Energy Analyzer, a nitrosamine-selective chemiluminescence detector (31). PEITC-NAC was analyzed as described by Chung *et al.* (30). Cotinine was determined by radioimmunoassay using antisera produced in rabbits (32). Creatinine was measured with a Kodak Ektachem 500 clinical chemistry analyzer.

Statistical Methods. Data are expressed as mean ± SD unless otherwise stated. The effects of watercress consumption on urinary metabolites were analyzed by a paired *t* test, and cor-

[4] S. S. Hecht, N. Trushin, and S.G. Carmella, unpublished data.

Table 1 Characteristics of study subjects and urinary excretion of PEITC-NAC during the watercress consumption period

Subject	Sex	Age	PEITC-NAC in urine (minimum amount of PEITC ingested in mg/24 h)[a]			
			Day 1	Day 2	Day 3	Day 4
1	M	34	36.8 (18.4)	69.3 (34.7)	39.1 (19.6)	1.62 (0.81)
2	M	27	15.7 (7.85)	36.5 (18.3)	10.4 (5.18)	8.98 (4.49)
3	F	26	21.8 (10.91)	37.5 (18.8)	41.4 (20.7)	NDe[b]
4	F	24	19.3 (9.64)	43.5 (21.8)	27.6 (13.8)	ND[c]
5	F	26	90.8 (45.4)	141 (70.5)	119 (59.7)	19.0 (9.5)
6	F	26	27.6 (13.8)	73.2 (36.6)	53.2 (26.6)	32.6 (16.3)
7	M	30	25.6 (12.8)	41.1 (20.6)	48.8 (24.4)	52.5 (26.3)
8	F	25	75.1 (37.6)	98.9 (49.4)	149 (74.5)	34.6 (17.3)
9	F	39	46.3 (23.1)	107 (53.5)	199 (99.5)	74.0 (37.0)
10	M	48	39.4 (19.7)	84.5 (42.3)	84.4 (42.2)	55.9 (28.0)
11	M	27	21.8 (10.9)	71.6 (35.8)	55.9 (28.0)	10.1 (5.1)
Mean ± SD		30.2 ± 7.4	38.2 ± 24.2 (19.1 ± 12.1)	73.1 ± 33.3 (36.6 ± 16.7)	75.3 ± 57.7 (37.7 ± 28.9)	28.9 ± 25.3 (14.5 ± 12.7)

[a] To convert to mmol/24 h, divide by 326 (PEITC-NAC) or 163 (PEITC).
[b] NDe, not detected.
[c] ND, not determined.

Table 2 Urinary NNAL plus NNAL-Gluc and cotinine in smokers who consumed watercress[a]

Subject	Cigarettes per day	Cotinine (μmol/24 h ± SD)[b]	NNAL plus NNAL-Gluc [nmol/24 h; mean ± SD or mean (individual values)]				*Difference [nmol/24 h (% change)]*		
			Period 1 (baseline)[c]	Period 2 (watercress consumption)[d]	Period 3 (Follow-up 1)[e]	Period 4 (Follow-up 2)[f]	Period 2 minus period 1	Period 3 minus period 1	Period 4 minus period 1
1	18.2 ± 2.2	49.4 ± 14.5	6.40 ± 2.10	8.30 (10.4, 6.21)	ND[g]	ND	1.90 (29.7)	ND	ND
2	16.7 ± 1.1	51.3 ± 7.90	5.80 ± 0.20	8.18 (7.57, 8.78)	9.92 (9.96, 10.4)	ND	2.38 (41.0)	3.52 (55.0)	ND
3	16.8 ± 1.1	37.5 ± 9.49	3.79 ± 1.08	4.66 (4.83, 4.48)	6.66 ± 1.44	ND	0.87 (23.0)	2.87 (75.7)	ND
4	15.0 ± 1.1	13.6 ± 4.34	4.18 ± 0.85	3.72 (3.66, 3.78)	2.30 (2.70, 1.89)	ND	−0.46 (−11.0)	−1.88 (−45.0)	ND
5	15.8 ± 0.4	51.4 ± 6.65	4.01 ± 0.46	5.64 (5.29, 6.00)	ND[g]	ND	1.63 (40.6)	ND	ND
6	9.5 ± 1.2	31.6 ± 7.61	0.799 ± 0.126	1.22 (1.16, 1.27)	ND[g]	ND	0.42 (52.7)	ND	ND
7	14.2 ± 1.1	23.6 ± 7.90	2.26 ± 0.50	1.26 (1.32, 1.20)	2.12 (1.48, 2.76)	0.913 ± 0.401	−1.00 (−44.2)	−0.14 (−6.2)	−1.53 (−59.6)
8	13.6 ± 0.7	29.3 ± 7.90	1.21 ± 0.44	2.69 (2.84, 2.54)	1.60 ± 0.29	1.43 ± 0.141	1.48 (122)	0.39 (32.2)	0.22 (18.2)
9	19.1 ± 1.7	33.5 ± 5.49	3.25 ± 0.74	5.57 (5.46, 5.68)	3.32 ± 0.74	3.71 ± 0.547	2.32 (74.1)	0.07 (2.2)	0.46 (14.2)
10	8.0 ± 1.4	17.4 ± 6.59	3.62 ± 1.72	3.99 (2.99, 4.99)	2.46 ± 0.77	2.56 ± 0.214	0.37 (10.2)	−1.16 (−32.0)	−1.06 (−29.3)
11	15.9 ± 1.0	14.6 ± 2.57	0.812 ± 0.117	1.06 (0.852, 1.27)	0.791 ± 0.118	1.07 ± 0.111	0.25 (30.5)	−0.02 (−2.6)	0.26 (31.8)
Mean ± SD	14.8 ± 3.4	32.1 ± 14.2	3.28 ± 1.88	4.21 ± 2.58	3.64 ± 3.08	1.94 ± 1.18	0.924 ± 1.12[h] (33.5 ± 43.2)	0.456 ± 1.85 (9.9 ± 41.6)	−0.294 ± 0.753 (−4.9 ± 38.2)

[a] Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables, except during the watercress consumption period. Twenty-four-h urine samples were collected as illustrated in Fig. 2. See "Materials and Methods" for details.
[b] On 3 baseline days, 3 watercress days, and 3 follow-up days. To convert to mg cotinine/24 h, multiply by 0.176.
[c] Twenty-four-h urine samples from 3 consecutive days.
[d] Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).
[e] Twenty-four-h urine samples from 2–3 consecutive days.
[f] Not determined.
[g] Significant compared to 0, $P < 0.01$ (paired t test).

relations were determined by calculation of Pearson's correlation coefficients (33).

Results

Characteristics of the study subjects and their urinary levels of a PEITC metabolite, PEITC-NAC, are summarized in Table 1. Watercress proved to be a good source of PEITC, confirming previous studies (28–30). On day 1 of watercress consumption, PEITC-NAC levels in urine ranged from 15.7 to 90.8 mg/24h, on day 2 from 36.5 to 141 mg/24h, and on day 3 from 10.4 to 199 mg/24h. Most of the PEITC-NAC was excreted by the end of day 3. The corresponding minimum amounts of PEITC ingested on each day are also shown in Table 1. The actual amount ingested cannot be determined without knowing the extent of metabolism of PEITC to PEITC-NAC, which has been estimated to range from 30 to 67% in humans (30).

Diet records indicated that all subjects complied with the protocol and avoided cruciferous vegetables or other sources of isothiocyanates during the study, except on the watercress consumption days. The urine of 7 subjects was analyzed for PEITC-NAC during the baseline and follow-up periods; PEITC-NAC was not detected.

Nine of the 11 subjects smoked between 10 and 20 cigarettes per day, as summarized in Table 2. Numbers of cigarettes smoked per day remained relatively constant, as requested in the protocol. Levels of NNAL plus NNAL-Gluc during the baseline period were also relatively constant for each smoker (mean coefficient of variation ± SD = 24.2 ± 11.3%).

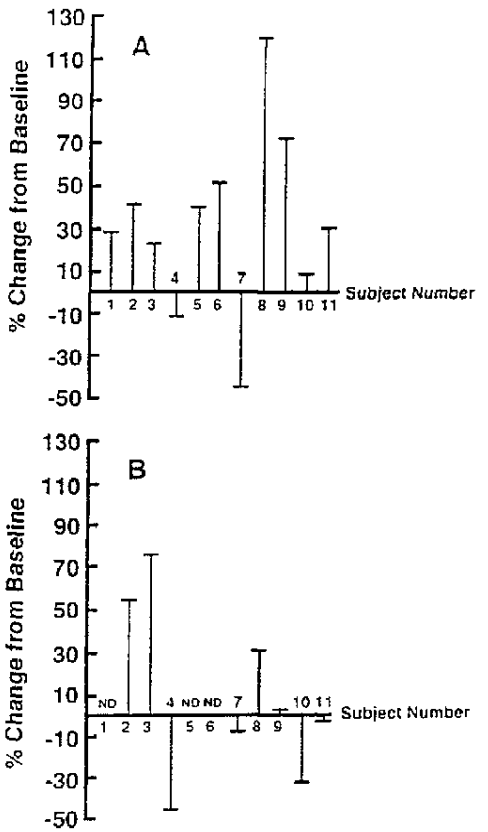

Fig. 3. The percentage of change in NNAL plus NNAL-Gluc excretion in the urine of each subject. *A*, days 2 and 3 of watercress consumption period compared to baseline. *B*, follow-up period 1 compared to baseline. ND, not determined.

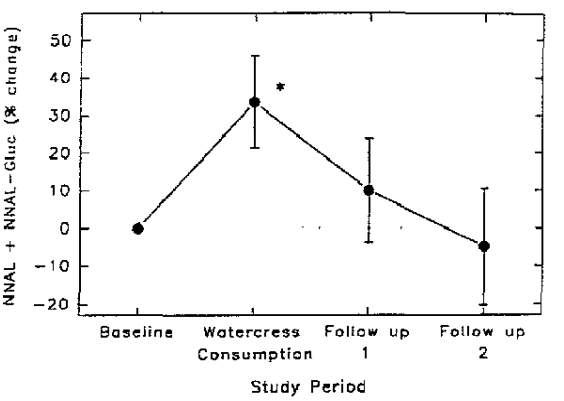

Fig. 4. Overall percentage of change (mean ± SE) in urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period, and in the follow-up periods, compared to the baseline period (n = 11 for baseline and watercress consumption periods, n = 8 for follow-up period 1, and n = 5 for follow-up period 2). *difference between the watercress consumption and baseline periods was significant ($P < 0.01$; see text). The differences between the follow-up and baseline periods were not significant. Data from Table 2.

Mean levels of urinary NNAL plus NNAL-Gluc in the baseline period, on days 2 and 3 of the watercress consumption period, and in the follow-up periods, are summarized in Table 2. In 7 of the 11 smokers, excretion of NNAL plus NNAL-Gluc increased on the second 2 days of the watercress consumption period compared to the baseline period. The amount was considered to have increased if the percentage increase was greater than 24.2%, the mean coefficient of variation in the baseline period. The percentage increases in these seven subjects ranged from 29.7 to 122% (mean ± SD = 55.8 ± 32.9%). Three subjects exhibited no change according to this criterion, and one had a decrease of 44.2%. The mean ± SD increase in NNAL plus NNAL-Gluc for all 11 subjects was 0.924 ± 1.12 nmol/24 h, which was significant ($P < 0.01$). In follow-up period 1, levels of NNAL plus NNAL-Gluc were determined in 8 of 11 subjects. They were higher than in the baseline period in 3 of the subjects, lower in 2, and unchanged in 3. The overall difference between follow-up 1 and baseline periods was 0.456 ± 1.85 nmol/24 h, which was not significant. Changes in NNAL plus NNAL-Gluc excretion in each subject in the watercress consumption period and follow-up period 1 compared to baseline are summarized in Fig. 3. Levels of NNAL plus NNAL-Gluc were determined in 5 subjects in follow-up period 2. Compared to baseline, they increased in 1, decreased in 2, and did not change in 2. The overall change compared to baseline was not significant. The composite percentage changes in the watercress consumption and follow-up periods are summarized in Fig. 4.

The NNAL plus NNAL-GLuc data were also expressed per mg creatinine, and the same calculations as described above were performed (Table 3). The mean ± SD increase on the second 2 days of the watercress consumption period compared to baseline was 0.454 ± 0.684 pmol/mg creatinine, which was significant ($P < 0.05$). The difference between follow-up period 1 and baseline was not significant. In follow-up period 2, there was a significant decrease in NNAL plus NNAL-Gluc compared to baseline ($P < 0.05$).

Although the maximum effects of watercress consumption on levels of NNAL plus NNAL-Gluc in urine were seen on days 2 and 3 of the watercress consumption period, the mean increase in levels of these metabolites on all 3 days of watercress consumption were still significant compared to the baseline period (mean increase, 0.626 ± 0.982 nmol/24 h; P = 0.03). Levels of NNAL plus NNAL-Gluc were also measured in the 24-h urine samples of 5 subjects on the day after the watercress consumption period. In 3 of the 5 subjects, the amount decreased compared to the preceding 2 days, by 34.9 ± 7.5%, whereas it was unchanged in the other 2 (data not shown).

The percentage of change from baseline in urinary levels of NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period was examined with respect to total urinary PEITC-NAC during days 1–3 of the watercress consumption period for each subject. These data are summarized in Fig. 5, which shows that these two variables correlated (r = 0.62; P = 0.04).

Insight on the potential effect of watercress consumption on UDP-glucuronosyl transferase activity can be obtained by considering levels of NNAL and NNAL-Gluc separately. These data for the first 3 periods of the study are summarized in Table 4. Six of the 11 subjects showed an increase in free NNAL on days 2 and 3 of the watercress consumption period compared to baseline, and 5 of these 6 individuals also had increases in

Table 3 Urinary NNAL plus NNAL-Gluc in smokers who consumed watercress[a]

Subject	NNAL plus NNAL-Gluc (pmol/mg creatinine; mean ± SD or mean (individual values))				Difference (pmol/mg creatinine (% change))		
	Period 1 (baseline)[b]	Period 2 (watercress consumption)[c]	Period 3 (follow-up 1)[d]	Period 4 (follow-up 2)[b]	Period 2 minus period 1	Period 3 minus period 1	Period 4 minus period 1
1	4.23 ± 0.15	5.78 (6.27, 5.29)	ND[e]	ND	1.55 (36.6)	ND	ND
2	3.62 ± 0.23	4.22 (3.95, 4.50)	5.17 (5.22, 5.12)	ND	0.60 (16.6)	1.55 (42.8)	ND
3	3.69 ± 0.70	4.48 (4.34, 4.62)	7.43 ± 0.43	ND	0.79 (21.4)	3.74 (101)	ND
4	3.83 ± 0.62	3.31 (3.21, 3.41)	3.08 (3.37, 2.78)	ND	−0.52 (−13.6)	−0.75 (−19.6)	ND
5	2.84 ± 0.31	3.42 (3.06, 3.78)	ND[e]	ND	0.58 (20.4)	ND	ND
6	0.914 ± 0.121	1.33 (1.23, 1.44)	ND[e]	ND	0.42 (45.5)	ND	ND
7	1.65 ± 0.09	1.06 (1.34, 0.77)	0.937 ± 0.580	0.632 ± 0.150	−0.59 (−35.8)	−0.71 (−43.2)	−1.02 (−61.8)
8	1.52 ± 0.31	2.41 (2.54, 2.29)	1.72 ± 0.23	1.29 ± 0.09	0.89 (58.6)	0.20 (13.2)	−0.23 (−15.1)
9	4.45 ± 0.43	5.73 (5.92, 5.54)	4.38 ± 0.08	3.99 ± 0.25	1.28 (28.8)	−0.07 (−1.6)	−0.46 (−10.3)
10	2.04 ± 0.57	2.09 (1.90, 2.28)	1.34 ± 0.09	1.49 ± 0.18	0.05 (2.5)	−0.70 (−34.3)	−0.55 (−30.0)
11	0.599 ± 0.186	0.547 (0.417, 0.676)	0.392 ± 0.060	0.474 ± 0.040	−0.052 (−8.7)	−0.207 (−35.0)	−0.125 (−21.7)
Mean ± SD	2.67 ± 1.37	3.13 ± 1.81	3.06 ± 2.44	1.57 ± 1.42	0.454 ± 0.684[f] (15.7 ± 27.7)	0.381 ± 1.55 (11.6 ± 47.4)	−0.478 ± 0.347[f] (−27.8 ± 20.4)

[a] Eleven smokers maintained constant smoking habits and avoided cruciferous vegetables except during the watercress consumption period. Twenty-four-h urine samples were collected as illustrated in Fig. 2. See "Materials and Methods" for details.
[b] Twenty-four-h urine samples from 3 consecutive days.
[c] Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).
[d] Twenty-four-h urine samples from 2–3 consecutive days.
[e] Not determined.
[f] Significant compared to 0; $P < 0.05$ (paired t test).

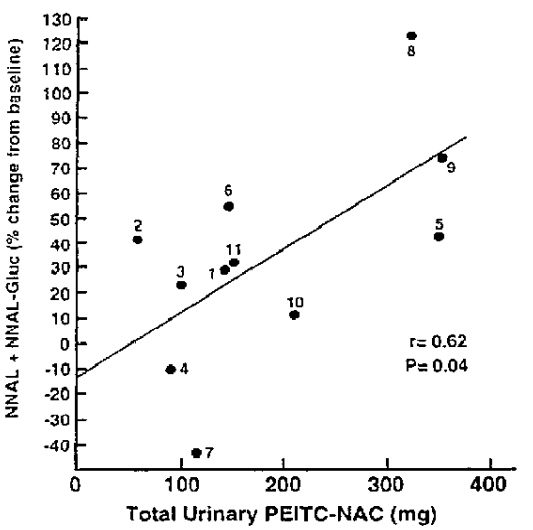

Fig. 5. Relationship between the percentage of change from baseline of urinary NNAL plus NNAL-Gluc on days 2 and 3 of the watercress consumption period and total urinary PEITC-NAC on days 1–3 of the watercress consumption period for the 11 smokers.

levels of NNAL-Gluc. Seven of the 11 subjects showed an increase in NNAL-Gluc on days 2 and 3 of the watercress consumption period, and 5 of these also had increased levels of NNAL. These changes are presented in summary form in Table 5. Consideration of Table 5 indicates that the NNAL-Gluc: NNAL ratio should have increased in two subjects (subjects 2 and 9) upon consumption of watercress. This was observed. The ratio for subject 2 increased from 3.3 at baseline to 5.2 on days 2 and 3 of watercress consumption, whereas for subject 9, the corresponding figures were 3.5 and 5.9. In the other 5 subjects with increased levels of NNAL plus NNAL-Gluc, there was no increase in ratio between baseline and days 2 and 3 of the watercress consumption period. Collectively, these data indicate that the major effect leading to increased NNAL plus NNAL-Gluc was an increase in the amount of available NNAL due to inhibition of another metabolic pathway. However, in two cases, UDP-glucuronosyl transferase activity may also have been induced.

The major contribution to increased urinary NNAL plus NNAL-Gluc was made by NNAL-Gluc because this is the more abundant of these two NNK metabolites. As shown in Tables 4 and 5, the 7 subjects with increased levels of NNAL-Gluc on days 2 and 3 of the watercress consumption period were the same 7 who had increased levels of NNAL plus NNAL-Gluc. The increase in urinary NNAL-Gluc on days 2 and 3 of the watercress consumption period (mean ± SD, 0.769 ± 0.968 nmol/24 h) was significant compared to baseline ($P < 0.02$).

Discussion

The results of this study support our hypothesis that watercress consumption increases urinary excretion of NNAL plus NNAL-Gluc in smokers. The mean increase in urinary NNAL plus NNAL-Gluc was significant in the watercress consumption period compared to baseline levels (Fig. 4). Urinary NNAL plus NNAL-Gluc levels decreased after the watercress consumption period. Changes in urinary NNAL plus NNAL-Gluc correlated with PEITC intake, as measured by PEITC-NAC levels in urine (Fig. 5). These data provide strong evidence that PEITC inhibits metabolic oxidation of NNK in smokers.

Studies in rats and mice have demonstrated that administration of PEITC results in inhibition of hepatic and pulmonary NNK α-hydroxylation and NNK-*N*-oxide formation (11, 23, 26–27). As shown in Fig. 1, this should result in accumulation and increased urinary excretion of NNAL and NNAL-Gluc. This was observed in a recently completed carcinogenicity

Table 4 Urinary NNAL and NNAL-Gluc in smokers who consumed watercress[a]

Subject	nmol/24 h			Difference [nmol/24 h (% change)]	
	Period 1 (baseline; mean ± SD)[b]	Period 2 [watercress consumption; mean (individual values)][c]	Period 3 [follow-up; mean ± SD or mean (individual values)][d]	Period 2 minus period 1	Period 3 minus Period 1
NNAL					
1	1.74 ± 0.78	2.49 (3.16, 1.82)	ND[e]	0.75 (43.1)	ND
2	1.34 ± 0.076	1.31 (0.994, 1.63)	1.75 (1.67, 1.83)	−0.03 (−2.2)	0.44 (30.6)
3	0.871 ± 0.228	1.20 (1.34, 1.05)	1.57 ± 0.344	0.329 (37.8)	0.699 (80.3)
4	0.925 ± 0.213	0.751 (0.832, 0.669)	0.559 ± 0.20	−0.174 (−23.2)	−0.366 (−39.6)
5	0.745 ± 0.105	1.28 (1.12, 1.43)	ND[e]	0.535 (71.8)	ND
6	0.221 ± 0.031	0.358 (0.342, 0.373)	ND[e]	0.137 (62.0)	ND
7	0.413 ± 0.111	0.138 (0.83, 0.193)	0.404 (0.184, 0.623)	−0.275 (−66.6)	−0.009 (−2.2)
8	0.356 ± 0.218	0.621 (0.609, 0.633)	0.318 ± 0.070	0.265 (74.4)	−0.038 (−10.7)
9	0.720 ± 0.226	0.805 (0.753, 0.886)	0.631 ± 0.111	0.085 (11.8)	−0.089 (−12.3)
10	0.646 ± 0.363	0.595 (0.519, 0.670)	0.480 ± 0.200	−0.051 (−7.8)	−0.166 (−25.7)
11	0.119 ± 0.042	0.206 (0.136, 0.276)	0.112 ± 0.041	0.087 (73.1)	−0.007 (−5.88)
Mean ± SD	0.736 ± 0.482	0.887 ± 0.670	0.728 ± 0.598	0.151 ± 0.303 (24.9 ± 46.4)	0.058 ± 0.344 (1.82 ± 37.6)
NNAL-Gluc					
1	4.66 ± 1.34	5.79 (7.19, 4.39)	ND	1.13 (24.2)	ND
2	4.46 ± 0.18	6.87 (6.58, 7.15)	8.17 (7.79, 8.55)	2.41 (53.9)	3.71 (83.2)
3	2.92 ± 0.87	3.46 (3.44, 3.43)	5.08 ± 1.13	0.54 (18.4)	2.16 (74.0)
4	3.26 ± 0.71	2.97 (2.83, 3.11)	1.86 ± 0.26	−0.29 (−8.9)	−1.40 (−42.9)
5	3.27 ± 0.36	4.37 (4.17, 4.57)	ND	1.10 (33.6)	ND
6	0.578 ± 0.096	0.858 (0.784, 0.932)	ND	0.280 (48.4)	ND
7	1.852 ± 0.395	1.12 (1.13, 1.12)	1.72 (1.30, 2.13)	−0.732 (−39.5)	−0.132 (−7.1)
8	0.860 ± 0.261	2.07 (2.23, 1.90)	1.28 ± 0.28	1.21 (141)	0.42 (48.8)
9	2.53 ± 0.518	4.75 (4.70, 7.80)	2.69 ± 0.63	2.22 (87.7)	0.16 (6.3)
10	2.97 ± 1.36	3.40 (2.47, 4.32)	1.98 ± 0.60	0.43 (14.5)	−0.99 (−33.3)
11	0.693 ± 0.087	0.858 (0.722, 0.994)	0.678 ± 0.132	0.165 (23.8)	−0.015 (−2.2)
Mean ± SD	2.55 ± 1.42	3.32 ± 2.01	2.93 ± 2.49	0.769 ± 0.968[f] (36.1 ± 47.9)	0.489 ± 1.676 (15.9 ± 47.5)

[a] See "Materials and Methods" and footnote *a* of Table 2 for details of protocol.
[b] Twenty-four-h urine samples from 3 consecutive days.
[c] Twenty-four-h urine samples from days 2 and 3 of the watercress consumption period (see Fig. 2).
[d] Twenty-four-h urine samples from 2–3 consecutive days.
[e] Not determined.
[f] Significant compared to 0; $P < 0.02$.

study in which rats consumed either control or PEITC-containing diet and were treated chronically with NNK in the drinking water.[4] Urinary levels of NNAL plus NNAL-Gluc were measured at two intervals during that study. The amounts of NNAL plus NNAL-Gluc were elevated in the PEITC-treated rats compared to control animals. Because technology to assess levels of α-hydroxylation and pyridine-*N*-oxidation metabolites of NNK in the urine of smokers is not yet available, we do not know whether one or both pathways are being inhibited by watercress in the present study; based on studies in rodents, however, it is likely that both α-hydroxylation and pyridine-*N*-oxidation have decreased in the smokers with increased levels of urinary NNAL plus NNAL-Gluc.

Experiments in rodents have shown that decreased α-hydroxylation is the major mechanism by which PEITC inhibits lung cancer induction by NNK (11, 23, 26–27). If similar mechanisms occur in humans, as this study indicates, PEITC may be an effective chemopreventive agent against NNK in smokers. Several epidemiological studies have shown that vegetable consumption protects against lung cancer in smokers, but the constituents of vegetables responsible for the protective effect have not been identified (19, 20). It is unlikely that any one constituent of vegetables is responsible. Our results suggest that PEITC may be one of the protective compounds. Despite the strong epidemiological evidence that vegetable consumption inhibits lung cancer, we are aware of no previous reports on the effects of vegetable consumption on lung carcinogen metabolism in humans. In related work, one study demonstrated that consumption of brussels sprouts and cabbage increased the metabolism of phenacetin in humans, whereas two others showed that consumption of brussels sprouts resulted in elevated levels of α-class glutathione *S*-transferase levels in human blood plasma (34–36). These effects are consistent with protective mechanisms proposed on the basis of animal studies (20, 21). A recent study showed no effect of a single 50-g portion of watercress on debrisoquine metabolism in humans (37).

An additional mechanism to explain increased levels of urinary NNAL-Gluc after watercress consumption would be induction of UDP-glucuronosyltransferase enzymes by PEITC or another constituent of watercress. In rats, PEITC treatment results in a small induction of hepatic UDP-glucuronosyltransferase (27). However, consideration of the data in Tables 4 and 5 indicates that in most smokers in our study, the major effect resulted from inhibition of other pathways, leading to increased levels of NNAL available for conjugation. Alternatively,

Table 5 Summary of changes in levels of NNAL, NNAL-Gluc, and NNAL plus NNAL-Gluc in smokers who consumed watercress[a]

Subject	NNAL	NNAL-Gluc	NNAL plus NNAL-Gluc
1	−	+	+
2	NC	+	+
3	−	NC	NC
4	NC	NC	NC
5	−	+	+
6	−	−	−
7	−	−	−
8	+	+	+
9	NC	+	+
10	NC	NC	NC
11	−	+	+

[a] From data in Tables 2 and 3. +, increase over baseline of greater than 30.8% for NNAL, 22.0% for NNAL-Gluc, and 24.2% for NNAL plus NNAL-Gluc. These percentages are the means of the coefficients of variation for these values for all subjects during the baseline period. −, decrease. NC, no change.

PEITC or another watercress constituent could induce carbonyl reductase activity or affect the rate of urinary excretion of NNK and its metabolites.

Ongoing studies of the effects of PEITC on NNK metabolism in the patas monkey indicate that the inhibition of oxidative metabolism is transitory and dependent on the presence of PEITC.[4] Changes in urinary NNK metabolite levels consistent with those described here were observed, but the effect decreased 24 h after PEITC administration. The results of the present study also indicate that the effects of PEITC are transient in some smokers because levels of NNAL plus NNAL-Gluc decreased on the day after the watercress consumption period and in the follow-up periods. However, in two smokers, levels of NNAL plus NNAL-Gluc were higher in follow-up period 1 than in the watercress consumption period. This requires further study.

The greatest increases in levels of urinary NNAL plus NNAL-Gluc were seen on days 2 and 3 of the watercress consumption period. This can be attributed to the experimental design. On day 1 of the watercress consumption period, urine collection began with the first morning void and thus contained NNK metabolites from smoking on the previous day. Therefore, the effect of watercress consumption on day 1 was diluted, although the overall increase in NNAL plus NNAL-Gluc in the watercress consumption period was still significant.

Although the aggregate data indicate a predictable effect of watercress consumption on NNK metabolism in smokers, increases of NNAL plus NNAL-Gluc during the watercress consumption period were observed in only 7 of the subjects, and there was considerable variation in the extent of the increase, ranging from 29.7 to 122% on days 2 and 3. Levels of NNAL plus NNAL-Gluc in 3 subjects did not change in the watercress consumption period, whereas they were markedly decreased in 1 subject. The effects that we observed are most likely due to inhibition by PEITC of human cytochromes P-450, including but not restricted to cytochrome P450 1A2 (12, 38). Levels of cytochrome P-450 enzymes will vary greatly among individuals, as will the metabolism of NNK (12, 13). Thus, the effects of PEITC and other constituents of watercress will also be different in each individual. If chemopreventive agents such as PEITC are to be used in intervention studies in smokers, it will be advisable to use biomarkers to select those smokers who are likely to be responsive.

In rats, the daily dose of PEITC that inhibited lung cancer induction by NNK was approximately 40 mg/kg body weight (240 μmol/kg) administered in the diet (23). NNK was given by s.c. injection at a daily dose of 1.76 mg/kg (8.5 μmol/kg). However, only about 0.3% (0.005 mg/kg or 0.025 μmol/kg) reaches the lung.[5] The PEITC:NNK molar ratio was approximately 9600, using the value for NNK dose in lung. In this study, the mean minimum amount of PEITC ingested on days 2 and 3 of the watercress consumption period was 37 mg/day, or approximately 0.5 mg/kg body weight (3 μmol/kg). This dose was only 1/80th as great as that used in the rat chemoprevention study. However, the daily dose of NNK was also much lower than in the rat study. Considering that urinary NNAL plus NNAL-Gluc may represent 50% of the dose of NNK, the mean daily dose of NNK would be approximately 6.4 nmol (0.085 nmol/kg), using the NNAL plus NNAL-Gluc values from the baseline period. Thus, the PEITC:NNK molar ratio in our study was approximately 35,000 (about 4 times as great as in the rat study). Although differences in routes of administration and possibly metabolism of PEITC and NNK in rats *versus* smokers preclude further comparisons, the concordance between the rat and human data are encouraging with respect to further development of PEITC as a chemopreventive agent.

Chemoprevention may be one way to decrease lung cancer risk in smokers who are addicted to nicotine and cannot stop smoking. The results of this study provide human data that bolster the growing body of evidence supporting further development of PEITC as a chemopreventive agent against lung cancer in smokers. Nevertheless, the potential beneficial effects of inhibition of oxidative metabolism of NNK may be offset by the increased levels of NNAL, which is a potent carcinogen. Moreover, the complexity of tobacco smoke must be kept in mind when considering chemoprevention strategies. The major lung carcinogens in tobacco smoke appear to be NNK and polynuclear aromatic hydrocarbons such as benzo[*a*]pyrene (39). Tobacco smoke also contains toxic agents, cocarcinogens, and tumor promoters. Whereas PEITC is a good inhibitor of NNK-induced lung carcinogenesis in animals, and the present results indicate that it also inhibits NNK metabolic activation in humans, it is not an effective inhibitor of lung tumor induction by benzo[*a*]pyrene (40, 41). Furthermore, suppressing agents that could reverse some of the damage done by tobacco smoke carcinogens and other toxic agents are needed, and PEITC has no activity of this type (42). It is likely that specifically designed mixtures of chemopreventive agents will be necessary for inhibition of lung cancer development in smokers.

Acknowledgments

We thank the participants for conscientiously following the protocol.

References

1. Hecht, S. S., and Hoffmann, D. The relevance of tobacco-specific nitrosamines to human cancer. Cancer Surv., *8*: 273–294, 1989.

2. Hecht, S. S., and Hoffmann, D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis (Lond.), *9*: 875–884, 1988.

3. Hecht, S. S., and Hoffmann, D. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, a nicotine-derived tobacco-specific nitrosamine, and cancer of the lung and pancreas in humans. *In:* J. Brugge, T. Curran, E. Harlow, and F. McCormick (eds.), Origins of Human Cancer: A Comprehensive Review, pp. 745–755. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1991.

4. Carmella, S. G., Akerkar, S., and Hecht, S. S. Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. Cancer Res., *53*: 721–724, 1993.

[5] M. E. Staretz and S. S. Hecht, unpublished data.

5. Hecht, S. S., Carmella, S. G., Foiles, P. G., Murphy, S. E., and Peterson, L. A. Tobacco-specific nitrosamine adducts: studies in laboratory animals and humans. Environ. Health Perspect., *99:* 57–63, 1993.

6. Hecht, S. S., Carmella, S. G., Foiles, P. G., and Murphy, S. E. Biomarkers for human uptake and metabolic activation of tobacco-specific nitrosamines. Cancer Res., *54* (Suppl.): 1912s–1917s, 1994.

7. Hecht, S. S. Recent studies on mechanisms of bioactivation and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific lung carcinogen. CRC Crit. Rev. Toxicol., in press, 1995.

8. Smith, T. J., Guo, Z., Thomas, P. E., and Yang, C. S. Kinetics and enzyme involvement in the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by rat lung and nasal mucosa. Carcinogenesis (Lond.), *13:* 1409–1414, 1992.

9. Guo, Z., Smith, T. J., Ishizaki, H., and Yang, C. S. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by cytochrome P450IIB1 in a reconstituted system. Carcinogenesis (Lond.), *12:* 2277–2282, 1991.

10. Guo, Z., Smith, T. J., Thomas, P. E., and Yang, C. S. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by inducible and constitutive cytochrome P450 enzymes in rats. Arch. Biochem. Biophys., *298:* 279–286, 1992.

11. Smith, T. J., Guo, Z., Li, C., Ning, S. M., Thomas, P. E., and Yang, C. S. Mechanisms of inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation in mouse by dietary phenethyl isothiocyanate. Cancer Res., *53:* 3276–3282, 1993.

12. Smith, T. J., Guo, Z. Y., Gonzales, F. J., Guengerich, F. P., Stoner, G. D., and Yang, C. S. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human lung and liver microsomes and cytochromes P-450 expressed in hepatoma cells. Cancer Res., *52:* 1757–1763, 1992.

13. Castonguay, A., Stoner, G. D., Schut, H. A. J., and Hecht, S. S. Metabolism of tobacco-specific *N*-nitrosamines by cultured human tissues. Proc. Natl. Acad. Sci. USA, *80:* 6694–6697, 1983.

14. Castonguay, A., Lin, D., Stoner, G. D., Radok, P., Furuya, K., Hecht, S. S., Schut, H. A. J., and Klaunig, J. E. Comparative carcinogenicity in A/J mice and metabolism by cultured mouse peripheral lung of *N*'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and their analogues. Cancer Res., *43:* 1223–1229, 1983.

15. Rivenson, A., Hoffmann, D., Prokopczyk, B., Amin, S., and Hecht, S. S. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived *N*-nitrosamines. Cancer Res., *48:* 6912–6917, 1988.

16. Hecht, S. S., and Trushin, N. DNA and hemoglobin alkylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its major metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in F344 rats. Carcinogenesis (Lond.), *9:* 1665–1668, 1988.

17. Morse, M. A., Eklind, K. I., Toussaint, M., Amin, S. G., and Chung, F-L. Characterization of a glucuronide metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its dose-dependent excretion in the urine of mice and rats. Carcinogenesis (Lond.), *11:* 1819–1823, 1990.

18. Hecht, S. S., Trushin, N., Reid-Quinn, C. A., Burak, E. S., Jones, A. B., Southers, J. L., Gombar, C. T., Carmella, S. G., Anderson, L. M., and Rice, J. M. Metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the Patas monkey: pharmacokinetics and characterization of glucuronide metabolites. Carcinogenesis (Lond.), *14:* 229–236, 1993.

19. Steinmetz, K. A., and Potter, J. D. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes & Control, *2:* 325–357, 1991.

20. Steinmetz, K. A., and Potter, J. D. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes & Control, *2:* 427–442, 1991.

21. Kelloff, G. J., Boone, C. W., Crowell, J. A., Steele, V. E., Lubet, R., and Sigman, C. C. Chemopreventive drug development: perspectives and progress. Cancer Epidemiol., Biomarkers & Prev., *3:* 85–98, 1994.

22. Morse, M. A., Wang, C-X., Stoner, G. D., Mandal, S., Conran, P. B., Amin, S. G., Hecht, S. S., and Chung, F-L. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA adduct formation and tumorigenicity in lung of F344 rats by dietary phenethyl isothiocyanate. Cancer Res., *49:* 549–553, 1989.

23. Morse, M. A., Amin, S. G., Hecht, S. S., and Chung, F-L. Effects of aromatic isothiocyanates on tumorigenicity, O^6-methylguanine formation, and metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. Cancer Res., *49:* 2894–2897, 1989.

24. Morse, M. A., Eklind, K. E., Amin, S. G., and Chung, F. L. Effect of frequency of isothiocyanate administration on inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary adenoma formation in A/J mice. Cancer Lett., *62:* 77–81, 1992.

25. Hecht, S. S. Chemoprevention by isothiocyanates. J. Cell. Biochem., *22* (Suppl.): 195–209, 1995.

26. Guo, Z., Smith, T. J., Wang, E., Eklind, K. I., Chung, F-L., and Yang, C. S. Structure-activity relationships of arylalkyl isothiocyanates for the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolism and the modulation of xenobiotic-metabolizing enzymes in rats and mice. Carcinogenesis (Lond.), *14:* 1167–1173, 1993.

27. Guo, Z., Smith, T. J., Wang, E., Sadrieh, N., Ma, Q., Thomas, P. E., and Yang, C. S. Effects of phenethyl isothiocyanate, a carcinogenesis inhibitor, on xenobiotic-metabolizing enzymes and nitrosamine metabolism in rats. Carcinogenesis (Lond.), *13:* 2205–2210, 1992.

28. Schultz, V. O-E. and Gmelin, R. Papierchromatographie der Senfolglucosid-Drogen. Z. Naturforsch., *7:* 500–506, 1952.

29. Tookey, H. L., VanEtten, C. H., and Daxenbichler, M. E. Glucosinolates. *In:* I. E. Liener (ed.), Toxic Constituents of Plant Stuffs, pp. 103–142. New York: Academic Press, 1980.

30. Chung, F-L., Morse, M. A., Eklind, K. I., and Lewis, J. Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. Cancer Epidemiol., Biomarkers & Prev., *1:* 383–388, 1992.

31. Carmella, S. G., Akerkar, S., Richie, J. P., Jr., and Hecht, S. S. Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. Cancer Epidemiol., Biomarkers & Prev., *4:* 635–642, 1995.

32. Haley, N. J., Axelrad, C. M., and Tilton, K. A. Validation of self-reported smoking behavior: biochemical analyses of cotinine and thiocyanate. Am. J. Public Health, *73:* 1204–1207, 1984.

33. Snedecor, G. W., and Cochran, W. G. Statistical Methods. Ed. 7. Ames, IA: Iowa State University Press, 1980.

34. Pantuck, E. J., Pantuck, C. B., Garland, W. A., Min, B., Wattenberg, L. W., Anderson, K. E., Kappas, A., and Conney, A. H. Stimulatory effect of brussels sprouts and cabbage on human drug metabolism. Clin. Pharmacol. Ther., *25:* 88–95, 1979.

35. Bogaards, J., Verhagen, H., Willems, M. I., van Poppel, G., and van Bladeren, P. J. Consumption of brussels sprouts results in elevated α-class glutathione *S*-transferase levels in human blood plasma. Carcinogenesis (Lond.), *15:* 1073–1075, 1994.

36. Nijhoff, W. A., Mulder, T. P. J., Verhagen, H., van Poppel, G., and Peters, W. H. M. Effects of consumption of brussels sprouts on plasma and urinary glutathione *S*-transferase class-α and -π in humans. Carcinogenesis (Lond.), *16:* 955–957, 1995.

37. Caporaso, N., Whitehouse, J., Monkman, S., Boustead, C., Issaq, H., Fox, S., Morse, M. A., Idle, J. R., and Chung, F-L. *In vitro* but not *in vivo* inhibition of CYP2D6 by phenethyl isothiocyanate (PEITC), a constituent of watercress. Pharmacogenetics, *4:* 275–280, 1994.

38. Smith, T. J., Guo, Z. Y., Guengerich, F. P., and Yang, C. S. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by human cytochrome P450 IA2 and inhibition by phenethyl isothiocyanate (PEITC). Proc. Am. Assoc. Cancer Res., *36:* 153, 1995.

39. Hoffmann, D., and Hecht, S. S. Advances in tobacco carcinogenesis. *In:* C. S. Cooper and P. L. Grover (eds.), Handbook of Experimental Pharmacology, pp. 63–102. Heidelberg, Germany: Springer-Verlag, 1990.

40. Lin, J-M., Amin, S., Trushin, N., and Hecht, S. S. Effects of isothiocyanates on tumorigenesis by benzo[*a*]pyrene in murine tumor models. Cancer Lett., *74:* 151–159, 1993.

41. Adam-Rodwell, G., Morse, M. A., and Stoner, G. D. The effects of phenethyl isothiocyanate on benzo[*a*]pyrene-induced tumors and DNA adducts in A/J mouse lung. Cancer Lett., *71:* 35–42, 1993.

42. Morse, M. A., Reinhardt, J. C., Amin, S. G., Hecht, S. S., Stoner, G. D., and Chung, F-L. Effect of dietary aromatic isothiocyanates fed subsequent to the administration of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone on lung tumorigenicity in mice. Cancer Lett., *49:* 225–230, 1990.